

Quantitative trait loci for insulin-like growth factor I, leptin, thyroxine, and corticosterone in genetically heterogeneous mice

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Harper, James M., Andrzej T. Galecki, David T. Burke, Stephen L. Pinkosky, and Richard A. Miller. Quantitative trait loci for insulin-like growth factor I, leptin, thyroxine, and corticosterone in genetically heterogeneous mice. *Physiol Genomics* 15: 44–51, 2003. First published July 15, 2003; 10.1152/physiolgenomics.00063.2003.—Genotype information was collected at 87 loci in a group of 1,108 UM-HET3 mice bred as the progeny of [BALB/cJ × C57BL/6J]F₁ mothers and [C3H/HeJ × DBA/2J]F₁ fathers, for which thyroxine (T₄), insulin-like growth factor I (IGF-I), and leptin levels had been measured at 4 and 15 mo of age. The data provided significant evidence for quantitative trait loci (QTL) modulating IGF-I levels on chromosomes 1, 3, 8, 10, and 17; for loci affecting T₄ on chromosomes 4, 15, and 17; and for leptin on chromosome 3. Fecal levels of corticosterone at 17 mo of age were influenced by a QTL on chromosome 1. Nine other gene/hormone associations reached a nominal $P < 0.01$, providing suggestive but not statistical evidence for additional QTL. QTL with an influence on a given hormone were in nearly all cases additive, with little or no evidence for epistasis. Of the 12 strongest QTL, 5 had effects that were age dependent, having more effect in 15-mo-old than in 4-mo-old mice in all but one case; the other QTL had effects that were apparently age-independent. These results show that the genetic controls over late-life hormone levels are complex and dependent on effects of genes that act both early and late in the life course.

epistasis; UM-HET3 mice; late-life hormone levels; age-specific

ALTHOUGH THERE IS A GROWING body of knowledge about genes whose inactivation leads to radical alterations in hormone levels (1, 3, 7, 18), much less is known about the ways in which common polymorphic alleles may contribute to differences in circulating hormone levels, either at early or at later stages in life. Previous studies in rodents (2, 8, 27, 32, 45), swine (14), and humans (31, 34, 37, 42) have successfully identified quantitative trait loci (QTL) for a variety of hormones, but these studies have focused on the identification of QTL linked to various disease states rather than on factors that modulate the natural variation in hormone levels.

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Most of the insulin-like growth factor I (IGF-I) in the circulation is produced by the liver in response to the pituitary peptide growth hormone (GH) and plays a prominent role in the determination of body size (9, 44), as well as being an important factor in the regulation of reproductive pathways (26, 39, 48, 54), energy balance (11, 22, 51, 52), and cell proliferation and death (6, 24, 25). In addition, IGF-I appears to play a role in the pathogenesis of common proliferative diseases (30).

Leptin is an adipocyte-derived protein hormone originally thought to function solely as a satiety signal (53), although it is now apparent that leptin can act both centrally and in the periphery to regulate a host of physiological processes. This includes growth, fertility, and glycemic control, as evidenced by leptin deficient *ob/ob* mice (36), as well as regulation of the hypothalamic-pituitary-adrenal (HPA) axis (19), bone growth (17), and T-cell immunity and the inflammatory response (16).

Thyroxine (T₄) and triiodothyronine (T₃) are synthesized in the thyroid gland using the amino acid tyrosine and the trace mineral iodine as building blocks and are key regulators of the basal metabolic rate (BMR) and heat production in mammals (13). Increased levels of either hormone will result in a corresponding increase in BMR and heat production via their effect on mitochondrial respiratory pathways (10, 13, 33). Moreover, the thyroid hormones are also key mediators of vertebrate growth and development (41, 44) and have been linked to oxidative defense mechanisms (12, 20, 21).

The glucocorticoids (GC) are a class of steroid hormones released during times of physiological and psychological stress, with corticosterone (CORT) being the primary bioactive GC in most species of rodent. GCs mobilize energy stores for immediate use (40) and by providing copious short-term energy may be invaluable for surviving sudden emergencies (46). Indeed, mild elevations in GC levels are beneficial for health (5). Nevertheless, chronic activation of this pathway does occur at the expense of other physiological processes and has been linked to significant impairments in growth (44), reproduction (35, 38, 50), and immune function (15).

In this study, 1,108 mice derived from a cross between CB6F1 females and C3D2F1 males were genotyped and sampled at the ages of 4, 15, and 17 mo to obtain blood or fecal material for the determination of IGF-I, leptin, T₄, and CORT levels. This population is the genetic equivalent of a large sibship and thus allows us to identify QTL that have detectably large effects on hormone levels. By measuring T₄, IGF-I, and leptin at both 4 and 15 mo, we were also able to determine whether the genetic effects noted might vary with age.

MATERIAL AND METHODS

Mice. The animals used in this study were of the UM-HET3 stock, bred at the University of Michigan as the offspring of (BALB/cJ × C57BL/6J)F₁ (CB6F1) females and (C3H/HeJ × DBA/2J)F₁ (C3D2F1) males; thus each mouse is genetically unique but shares ~50% of its genes, on average, with every other mouse in the group. A total of 1,108 (826 females, 282 males) virgin mice were used.

All mice were weaned at 3 to 4 wk of age, housed in same-sex cages, and given free access to food and water for the duration of the study. To ensure the specific pathogen-free status of the study population, groups of sentinel mice were exposed to spent bedding from the study population on a quarterly basis and were later evaluated serologically for the presence of specific viral and bacterial pathogens. The animals were also examined for pinworm. All test results were negative over the course of the study.

Mice were entered into the study in cohorts of ~30 per month with all mice housed in the Cancer Center and Geriatrics Center Building at the University of Michigan Medical Center. For the determination of serum hormone levels, all mice were immunized with erythrocytes at the ages of 4 and 15 mo as part of another protocol and bled by tail venipuncture 2 wk after each immunization to assess antibody production as an index of their immunoresponsiveness. The remaining serum samples were used for the quantification of circulating T₄, leptin, and IGF-I levels in this study. In addition, at 12 mo of age skin biopsy samples were obtained under brief Metofane anesthesia as part of another protocol, and 1–3 fecal pellets were collected from approximately one-third ($n = 321$ females) of the mice at 17 mo of age for the quantification of fecal corticosteroid levels for use in this study. Fecal samples were collected from each mouse for five consecutive days to control for the possibility of significant day-to-day variation in circulating CORT levels due to the influence of the estrus cycle (4). The mean 5-day fecal corticosteroid level for each mouse was used for the analysis.

Hormone measurements. Serum samples for hormone measurements were taken by tail venipuncture between the hours of 7 and 11 AM and stored at -70°C for up to 3 yr prior to assay. Serum T₄ levels were determined using a monoclonal solid-phase radioimmunoassay (RIA) kit (ICN Pharmaceuticals, Costa Mesa, CA) run at one-quarter volume according to the manufacturer's instructions. Each sample was assayed in duplicate and diluted up to 1:7 with phosphate-buffered saline (PBS) if necessary to achieve adequate sample volume. Serum IGF-I levels were quantified via a double-antibody RIA kit (Diagnostic Systems Laboratories, Webster, TX) run at one-quarter volume according to the manufacturer's instructions. Prior to assay, 10 μl of serum from each individual was subjected to an acid-ethanol extraction procedure using the materials provided in the kit. All samples were assayed in duplicate. Serum leptin levels were quanti-

fied with a double-antibody RIA kit (Linco Research, St. Charles, MO) according to the manufacturer's instructions, except that all volumes were reduced by a factor of 4. Each sample was assayed in duplicate using dilutions up to 1:10.4 with PBS if necessary to achieve adequate sample volume. The inclusion of two pooled serum controls run in each assay ($n = 13$) indicated that the mean ($\pm\text{SD}$) intra-assay coefficients of variation (CV) were $6.95 \pm 5.55\%$, $5.89 \pm 4.75\%$, and $6.12 \pm 4.53\%$ for T₄, IGF-I, and leptin, respectively, and that the inter-assay coefficient CV was less than 25% for all. The lower limits of detection were 150 ng/ml, 0.2 ng/ml, and 0.625 $\mu\text{g}/\text{dl}$ for IGF-I, leptin and T₄, respectively.

Fecal corticosteroid levels were quantified with a double-antibody ¹²⁵I-corticosterone RIA kit (ICN Biomedicals) after hormone extraction using an ethanol-based procedure as described in (23). This procedure has been validated previously for use in multiple species of rodent as a specific, precise, and accurate integrated measure of circulating corticosteroid concentrations (23). All samples were assayed in duplicate, and the assay was performed according to the manufacturer's instructions at one-quarter volume. The 25 ng/ml standard provided with the kit was diluted 1:2 with steroid diluent (provided with the kit) to allow the inclusion of a 12.5 ng/ml standard (the lower limit of detection for this assay). Corticosteroid concentrations are presented as nanograms corticosteroids per gram dry weight of feces. As an indicator of the day-to-day variation in fecal CORT levels over the 5-day sampling period, the intra-individual CV of the 5-day mean fecal CORT measure was calculated for each mouse. The result of this analysis indicated that fecal CORT levels typically varied by ~36% within a five-day sampling period (mean \pm SD; $35.95 \pm 15.62\%$). In addition, the inclusion of two pooled fecal extracts in each assay ($n = 20$) indicated that the mean ($\pm\text{SD}$) intra-assay CVs were $3.73 \pm 3.62\%$ and $8.45 \pm 7.31\%$ for the low and high control, respectively. The interassay CV was 8.7% for the low control and 18.8% for the high control.

Genotyping. Genomic DNA was isolated from a 1-cm section of tail collected from 4-wk-old individuals using a phenol-extraction method. The DNA concentration, ability to sustain PCR amplification, and electrophoretic size distribution for each of the preparations were tested prior to simple-sequence length polymorphism (SSLP) genotyping using an ALFexpress automated sequence analyzer (Pharmacia, Piscataway, NJ) using previously described methods (28). Primer pairs were either purchased from Research Genetics or were synthesized locally (University of Michigan Molecular Biology Core Facility). Polymorphic loci were selected using data provided by the Mouse SSLP Database (Whitehead/MIT Center for Genome Research, Cambridge, MA) or the Mouse Genome Database (Jackson Laboratory, Bar Harbor, ME). Chromosomal localization and marker order were calculated using the MapMaker QTX software package (Whitehead Institute, Cambridge, MA).

Statistical analyses. A single point genome-wide search was performed for each of the seven hormone measures to detect QTL that may be associated with each measure. To make the analysis consistent for all partially and fully informative markers, four-way informative markers were split into two sets of bi-allelic markers that were informative for either the maternally or paternally transmitted alleles. One-way ANOVA models, with each hormone measure as the dependent variable and a bi-allelic marker as the independent variable, were used to perform a genome-wide search for all of the 164 bi-allelic markers. The statistical significance for each marker-trait combination was calculated empirically

Table 1. *Hormone levels in young adult and middle-aged UM-HET3 mice*

Hormone	Age, (mo)	Female		Males	
			<i>n</i>		<i>n</i>
IGF-I, ng/ml	4	681(578–798)	725	798(685–919)	246
	15	734(633–845)	714	853(714–1,040)	223
Leptin, ng/ml	4	3.1(2.1–4.4)	664	2.9(1.9–4.6)	202
	15	5.3(3.5–9.2)	645	5.0(3.2–8.8)	176
T ₄ , µg/dl	4	5.9(4.8–7.1)	744	6.0(5.0–7.2)	252
	15	5.1(4.1–6.2)	745	4.6(3.9–5.8)	218
CORT, ng/ml	17	71.1(51.5–93.2)	321	ND	

Values are median, with interquartile range in parentheses; T₄, thyroxine; CORT, corticosterone; ND, not done.

using a permutation-based technique. This allows the generation of an “experiment-wise” acceptance criterion that takes multiple comparisons into account to avoid type I error inflation. A null distribution for permutation analysis was generated based on 1,000 random shuffles of the original phenotypic data. In addition, genetic marker- and age-related interactions, as well as the amount of variability of each hormone explained by genetic effects, were studied using ANOVA.

RESULTS

Circulating hormone levels. Table 1 summarizes the circulating IGF-I, leptin, and T₄ levels observed in this population of 4- and 15-mo-old male and female UM-HET3 mice, as well as the 5-day fecal corticosteroid level for 17-mo-old UM-HET3 females only. A repeated measures ANOVA was used to test the effect of age and sex on these hormone levels and revealed that all three of the serum hormones were altered by age. The sex effect was significant only for IGF-I (males with higher levels than females). The interaction term in the repeated measures ANOVA [age × sex] showed that the effect of age on leptin and T₄ levels was more substantial in males than in females for leptin ($P = 0.02$) and for T₄ ($P = 0.04$), but there was no significant interaction for IGF-I.

Detection of QTL. Genome-wide scans evaluated 87 SSLP markers known to discriminate among the four inbred progenitor strains. Of the 87 marker loci analyzed, 77 were informative for all of the progenitor strains, 10 were informative for either the maternal or paternal alleles only (8 maternal, 2 paternal), and 2 were X-linked. Considering maternally-inherited and paternally-inherited markers independently, the genotype data set included 164 bi-allelic markers. The average distance between markers in the scan is 15–20 cM, depending on how sex-specific recombination frequencies are treated, and excluding the region between the most distal marker and the telomere for each chromosome. About 5% of the genome lies more than 20 cM from a marker used in the survey.

Using a permutation method to derive experiment-wise significance criteria, we found 12 significant gene/trait associations, as shown in Table 2. IGF-I levels were affected by loci on chromosomes 1, 3, 8, 10, and 17; leptin levels were affected by a locus on chromosome 3; T₄ levels were modulated by loci on chromosomes 4, 15, and 17; and fecal CORT levels were influenced by a locus on chromosome 1. Each marker was only associated with a single trait measured at a specific age with one exception: *D4Mit155* was significantly associated with serum T₄ levels in both 4- and 15-mo-old individuals. The marker *D17Mit46* is also listed twice in Table 2, but it is the maternal allele that is associated with alterations in IGF-I levels (at 15 mo), and the paternal allele associated with differences in T₄ levels in 4-mo-old mice. Four significant markers, i.e., *D3Mit227*, *D3Mit86*, *D15Mit63*, and *D1Mit206*, are not shown in Table 2, because they are each within 16 cM of another marker with a stronger level of association with the trait concerned and thus are unlikely to represent an independently segregating allele with functional effect.

There is evidence for sex-specific influences on many age-sensitive traits [e.g., bone geometry (32), obesity (49), behavior (43)]. We therefore conducted the ge-

Table 2. *Significant gene/trait associations with experiment-wise $P < 0.05$*

Marker	Position		Inheritance	Hormone (Age, mo)	<i>n</i>	Effect (SE)	Experiment-wise <i>P</i> -Value	Comparison
	kbp	cM						
<i>D1Mit206</i>	174,305	95.8	Mat	IGF-I (4)	941	44(12)	0.05	C > B6
<i>D10Mit230</i>	90,393	49.0	Pat		929	57(12)	<0.001	C3 > D2
<i>D17Mit185</i>	67,586	40.6	Mat		951	48(12)	0.01	C > B6
<i>D3Mit25</i>	80,353	38.3	Mat	IGF-I (15)	903	54(13)	0.01	B6 > C
<i>D8Mit51</i>	88,132	41.0	Mat		896	56(13)	0.01	B6 > C
<i>D17Mit46</i>	24,558	10.0	Mat		895	63(13)	<0.001	C > B6
<i>D3Mit127</i>	143,698	70.3	Pat	Leptin (4)	801	0.8(0.2)	0.01	C3 > D2
<i>D4Mit155</i>	100,282	49.6	Pat	T ₄ (4)	943	0.5(0.1)	0.01	C3 > D2
<i>D17Mit46</i>	24,558	10.0	Pat		907	0.5(0.1)	0.01	C3 > D2
<i>D4Mit155</i>	100,282	49.6	Pat	T ₄ (15)	909	0.4(0.1)	0.02	C3 > D2
<i>D15Mit100</i>	51,347	21.0	Mat		923	0.4(0.1)	0.001	C > B6
<i>D1Mit105</i>	161,635	80.0	Pat	CORT (17)	249	19(4)	0.001	D2 > C3

“Inheritance” indicates whether the allele was either maternally (Mat) or paternally (Pat) derived. The estimated effect of each marker is the absolute difference between mean levels of the indicated trait in the two groups of mice differing at the indicated locus. “Comparison” indicates which allele results in the larger value of a particular trait. When the genome scan showed two or more closely linked markers with significant associations to the same hormone, only the one with the stronger association is listed here. B6, C57BL/6J mice; C, BALB/cJ mice; C3, C3H/HeJ mice; D2, DBA/2J mice.

Table 3. *Post hoc analysis of markers listed in Table 2*

Marker	Inheritance	Hormone (Age, mo)	Effect (SE)	Nominal <i>P</i> Value	Comparison
<i>D1Mit206</i>	Mat	IGF-I (4)	44 (12)	<0.001*	C > B6
	Mat	IGF-I (15)	39 (13)	0.003	C > B6
<i>D3Mit127</i>	Pat	Leptin (4)	0.9 (0.2)	<0.001*	C3 > D2
	Pat	Leptin (15)	1.1 (0.4)	0.004	C3 > D2
<i>D3Mit227</i>	Mat	IGF-I (15)	51 (13)	<0.001*	B6 > C
<i>D8Mit51</i>	Mat	IGF-I (4)	39 (12)	0.001	B6 > C
	Mat	IGF-I (15)	56 (13)	<0.001*	B6 > C
	Mat	T ₄ (4)	0.3 (0.1)	0.002	C > B6
<i>D10Mit230</i>	Pat	IGF-I (4)	57 (12)	<0.001*	C3 > D2
<i>D15Mit100</i>	Mat	IGF-I (15)	41 (13)	0.002	C > B6
	Mat	T ₄ (4)	0.4 (0.1)	0.001	C > B6
	Mat	T ₄ (15)	0.5 (0.1)	<0.001*	C > B6

*Trait with experiment-wise $P < 0.05$ from Table 2.

nome-wide QTL analysis twice: first with the data pooled across sex, and then a second time with sex included as an independent variable in the statistical model. Each of the markers listed in Table 2 remained significant at experiment-wise $P < 0.05$ in the sex-adjusted model, and therefore we report only the results obtained using the pooled dataset.

In addition to the statistically significant associations shown in Table 2, we have compiled a Supplementary Table listing each of the markers for which the genome scan provided suggestive evidence at a less conservative criterion of $P < 0.20$. This Supplementary Table can be viewed at <http://www-personal.umich.edu/~millerr/Harper%20suppl%20table%201.htm>, and is also available at the *Physiological Genomics* web site.¹

It is possible polymorphic alleles might modify more than one of the tested hormones but that only one of the associations is sufficiently strong to reach the experiment-wise significance threshold. To examine this question, further analysis was performed to determine whether any of the QTL listed in Table 2 had an influence on the level of the other hormones measured in this study. We used a Bonferroni-corrected P value, $P = 0.004$, as our criterion for rejecting the null hypothesis that the alleles listed in Table 2 had no effect on any of the other hormones. The results of this post hoc analysis are presented in Table 3, which also includes the original associations from Table 2 for completeness. Overall, these results suggest that some of the chromosomal segments containing significant QTL show associations with the production or metabolism of multiple hormones or of the same hormone at more than one age. It is of particular interest to note that the B6 allele on chromosome 8 is associated with high IGF-I and low T₄ levels, whereas the B6 allele on chromosome 15 is associated with low levels of both of these hormones, suggesting that the two alleles may affect different aspects of the hormonal response. Further work would be needed to determine whether the

associations noted in Table 4 reflect the actions of single QTL or of multiple QTL linked to one another on the indicated chromosomes, and to investigate the extent to which changes in one hormone might bring out secondary, compensatory alterations in the levels of other hormones in our test battery.

Tests for gene-gene interactions. For each of the hormones for which two or more QTL reached the experiment-wise significance criterion (Table 2), we explored the question of whether the genetic effects were additive or were instead conditional on inheritance at the other influential loci. We used two different methods. In the first, less formal approach, the sum of the individual effects for each of the significant markers was compared with the pooled genetic effect, i.e., to the difference in hormone levels between the two composite genotypes that differed the most for the hormone measure in question. The top row of Table 4, for example, shows these statistics for the three QTL with effects on IGF-I levels at 4 mo of age. Each of these three loci has an individual effect (shown in Table 2), and the sum of these effects is 149 ng/ml. The three loci define eight possible genotypic combinations, and the difference in mean IGF-I levels between the two groups with highest and lowest levels comes to 145 ng/ml, a value similar to the sum of the individual effects. We thus see no evidence for non-additivity in this or indeed in any of the other hormones evaluated in this way, as illustrated in Table 4.

In the second approach, a full-factorial ANOVA was performed that used all of the significant markers as factors in the model, and the P value of the interaction term, $p(I)$, that involved all of the significant markers was determined. A nonsignificant interaction term is interpreted as no genetic interaction, or epistatic effect, for the markers tested. The results of these analyses are also presented in Table 4 and indicate that there was no significant evidence for epistasis for any of the hormones tested, with the possible exception of serum IGF-I levels in 15-mo-old mice. In this case, the second-order interaction between *D8Mit51* and *D3Mit25* was significant at $P = 0.05$. However, it is possible this finding is due simply to chance. Neither the third-order interaction, nor the two remaining second-order interactions, are significant at $P = 0.10$, and

¹The Supplementary Material for this article (a table listing each of the markers for which the genome scan provided suggestive evidence at a less conservative criterion of $P < 0.20$) is available online at <http://physiolgenomics.physiology.org/cgi/content/full/00063.2003/DC1>.

Table 4. Tests for additivity and epistatic genetic effects on serum hormone levels

Hormone (Age, mo)	Markers	Sum of Individual Effects	Pooled Effect	p(I)
IGF-I (4)	<i>D1Mit206, D10Mit230, D17Mit185</i>	149	145	0.22
IGF-I (15)	<i>D3Mit25, D8Mit51, D17Mit46</i>	173	187	0.05
Leptin (15)	<i>D11Mit289, D13Mit64</i>	2.6	2.6	0.64
T ₄ (4)	<i>D4Mit155, D17Mit46</i>	1	0.9	0.42
T ₄ (15)	<i>D4Mit155, D15Mit100</i>	0.9	0.9	0.78

Units for each of the hormone measures are as follows: IGF-I, ng/ml; leptin, ng/ml; T₄, µg/dl; and CORT, ng/ml.

the sum of the estimated and pooled genetic effects are virtually identical (173 vs. 187 ng/ml). Figure 1 illustrates this additivity for serum IGF-I levels in 15-mo-old mice. For each of the three significant markers, the median concentration of serum IGF-I is shown with the effect of the B6 or C allele at each of the three loci. At *D3Mit25* and *D8Mit51* inheritance of the B6 allele is associated with an increase in serum IGF-I, whereas at *D17Mit46* inheritance of the C allele has the same effect. For mice that inherit the B6 allele at *D3Mit25* and *D8Mit51* and the C allele at *D17Mit46*, their serum IGF-I concentration is 187 ng/ml greater than those who inherited the opposite alleles at these loci (Fig. 1).

We also determined what proportion of the variance observed for each of the seven hormone measures was attributable to the genetic markers found to be statistically significant for each hormone. These results indicate that the proportion of attributable variance ranges from 2 to 8%, depending upon the measure. The net effect, calculated as the difference in mean hormone level between the highest and lowest genotypic group, was ~15–20% of the mean level for IGF-I, leptin, and T₄.

Age-related effects on hormone measures. Circulating levels of three of the hormones were measured at two

ages (4 and 15 mo) in most of the mice. This allowed us to test whether the effect of a given allele on circulating IGF-I, leptin, or T₄ levels varies with age. For a more systematic exploration of this issue, we performed a repeated measures ANOVA for each of the 10 markers with an experiment-wise $P < 0.05$, as well as for the two leptin QTL for which experiment-wise $P < 0.10$. For most of the markers the [age × genotype] interaction term failed to reach statistical significance, providing no evidence for an age-specific effect. There were five instances, however, in which the strength of the genetic effect was indeed age sensitive. In four of five of these cases, the effect of the genetic polymorphism is more dramatic at the older age than it is in young mice. In particular, for IGF-I the markers *D3Mit25* and *D17Mit46*, and for leptin the markers *D11Mit289* and *D13Mit64*, all had a significant effect on circulating hormone levels in 15- vs. 4-mo-old mice. On the other hand, the marker *D10Mit230* had a greater effect on circulating IGF-I levels in 4- vs. 15-mo-old mice. Figure 2 shows two examples of situations in which the effect of an allele is equally apparent at both ages tested (IGF-I, *D8Mit51*; leptin, *D3Mit127*) and two others for which the allele effect is apparent only at the older age (IGF-I, *D3Mit25*; leptin, *D11Mit289*). In this example, for IGF-I $p(I) = 0.40$ for the marker *D8Mit51*, whereas $p(I) = 0.01$ for the marker *D3Mit25*. This suggests that the effect of *D3Mit25* on circulating IGF-I levels changes with age but that *D8Mit51* does not; a suggestion that is clearly borne out by the illustration of these effects in Fig. 2.

DISCUSSION

Using a population derived from a four-way breeding scheme, we have demonstrated 12 significant QTL that regulate serum IGF-I, leptin, T₄, and/or fecal CORT levels in mice using a genome-wide scan. Ten of these QTL were associated with serum IGF-I, leptin, and T₄ levels at one of two ages (4 and 15 mo), one QTL was associated with the serum T₄ level in both 4- and 15-mo-old mice, and one QTL was associated with fecal CORT levels in 17-mo-old female mice (the only age at which CORT was tested). In addition, we found suggestive evidence for nine other QTL (experiment-wise $0.05 < P < 0.20$) with effects on serum IGF-I, leptin, and T₄. Moreover, for each of the QTL with an experiment-wise $P < 0.10$, post hoc analyses revealed that the genetic effects of each of these loci was additive and that they explained ~2–8% of the variation in hormone levels at each of the ages tested.

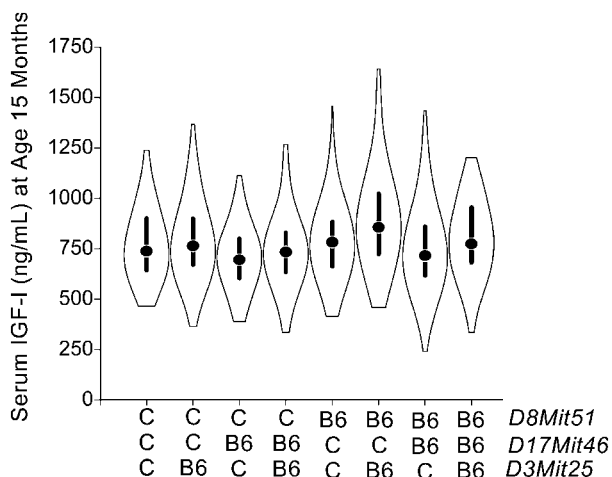


Fig. 1. Additive genetic effects of the loci linked to *D8Mit51*, *D17Mit46*, and *D3Mit25* on serum insulin-like growth factor I (IGF-I, ng/ml) levels in 15-mo-old UM-HET3 mice shown for each of the eight possible genotypic allelic combinations at these three loci. In each plot, the circle represents the median; the vertical lines show the 25th and 75th percentiles, and the density trace shows the distribution of the values for each genotype. C, BALB/cJ mice; B6, C57BL/6J mice.

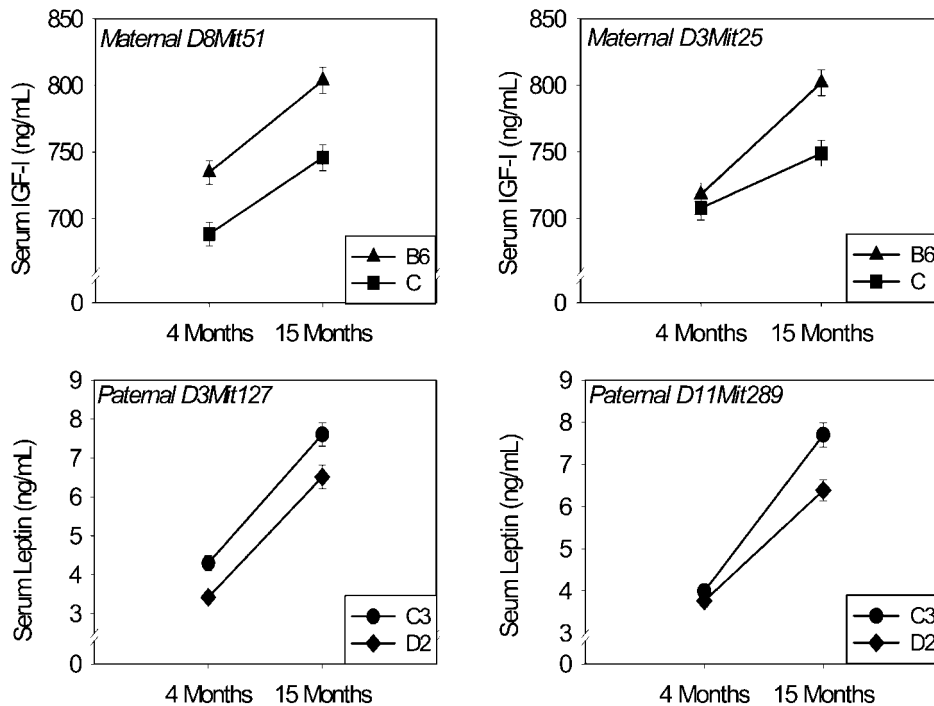


Fig. 2. Illustrations of age-independent (left) and age-dependent (right) gene/trait associations. For the loci *D8Mit51* (top left) and *D3Mit25* (top right) the effect of either the B6 vs. C allele on serum IGF-I levels is shown for the 4-mo and 15-mo time points. For *D8Mit51* the association does not change over time [2-way ANOVA, $p(I) = 0.35$], but it does change with time [2-way ANOVA, $p(I) = 0.02$] for *D3Mit25*. For serum leptin levels, the effect of C3 or D2 alleles at the loci *D3Mit127* (bottom left) and *D11Mit289* (bottom right) is shown for 4- and 15-mo-old mice. For *D3Mit127* the association does not change over time [2-way ANOVA, $p(I) = 0.62$], but there is an age-related effect [2-way ANOVA, $p(I) = 0.01$] for *D11Mit289*. C3, C3H/HeJ; D2, DBA/2J.

Previous studies have also identified QTL for serum IGF-I (8, 45) and leptin (8) in mice. There was, however, little overlap between the QTL identified in the previous studies and the QTL identified here. For example, the chromosome 14 QTL for serum leptin identified by Brockman and colleagues (8) was documented using a cross between DBA.2 mice and mice of a stock, D6Ui, that had previously been selected for extreme growth rate. The significant and suggestive QTL for serum leptin identified in this study reside on chromosomes 2, 3, 11, and 13, but it is difficult to compare these results to Brockman's findings because of the differences in the genetic backgrounds of the stocks examined.

Brockman and colleagues (8) also reported that there were two QTL for serum IGF-I levels in their stock mice: one on chromosome 10 and the other on chromosome 18. In contrast, Rosen and colleagues (45) reported a significant association between serum IGF-I levels and QTL on chromosomes 6, 10, and 15 in a population of 4-mo-old female (C57BL/6 \times C3H/He)F2 mice. Interestingly, the QTL identified on chromosome 10 in each of these studies map to the same region as the one identified by us and in each case are in close proximity to the mouse *Igf1* gene. A recent study (47) has shown that QTL regulating levels of gene expression are often linked to the structural genes in question and are often clustered in proximity to other QTL regulating similar physiological systems. It thus seems plausible that this area of mouse chromosome 10 marked by *D10Mit230* in our study could contain multiple genes with effects on IGF-I levels, perhaps including polymorphic regions with effects on transcription of *Igf1* per se. Similarly, the IGF-I QTL identified on chromosome 15 by Rosen et al. (45) maps to approxi-

mately the same region as a suggestive QTL on chromosome 15 identified in this study.

To the best of our knowledge this study is the first to identify any QTL for either T_4 or CORT in mice, although two QTL for serum cortisol levels have been reported for humans (42). Interestingly, both of these human QTL are on human chromosome 1q and map to a region that is orthologous to that containing the locus *D1Mit105* in mice, linked in our study to mouse CORT levels (http://www.informatics.jax.org/reports/homologymap/mouse_human.shtml).

For several of these loci, likely candidate genes can readily be identified. In addition to the linkage of *D10Mit230* to the mouse *Igf1* locus mentioned earlier, we note that the IGF-I QTL linked to *D17Mit46* is in close proximity to the IGF-II receptor (*Igf2r*) gene. Furthermore, the markers *D4Mit155* and *D15Mit100* are included in regions on each of the two chromosomes that contain genes integral to the metabolic control of thyroid hormones: type I iodothyronine deiodinase (*Dio1*) on chromosome 4; and the thyroglobulin releasing hormone receptor (*Trhr*) and thyroglobulin (*Tgn*) on chromosome 15. Each of the chromosomal regions identified by this low-resolution study, however, may well contain additional genes with direct or indirect effects on hormone levels, and additional work will be needed to determine the specific locus (or loci) responsible for the effects documented in Table 2.

Each of the 12 loci with an experiment-wise $P \leq 0.10$ was tested formally to see whether we could exclude the hypothesis of age independence. In five cases we were able to reject the null hypothesis, and found that in four of these five cases the genetic effect was stronger in older than in young animals. For example, the loci at *D3Mit25* and *D17Mit46* had only negligible

effects on the serum level of IGF-I in 4-mo-old mice but a very large effect in 15-mo individuals. However, the opposite relationship was observed for the locus *D10Mit230*, which had an appreciable effect on serum IGF-I levels in 4-mo-old mice but little effect in the 15-mo-old individuals. Thus five of the QTL seem to have an age-sensitive effect, and four of these only manifest their effect later in life. It will be interesting to learn whether these affect different metabolic pathways than do loci whose effect on hormone levels is established in young adults and unvarying thereafter.

In a previous study, mice generated using an identical breeding scheme indicated that there were multiple QTL for the regulation of T-cell subset distributions in this stock and that the effects of these loci could either be stable or age specific (29), similar to what we observed in this study. Interestingly, the QTL at *D13Mit57* identified by Jackson and colleagues (29) for the regulation of CD4 levels in 18-mo-old mice and the QTL at *D13Mit64* identified in this study for the regulation of serum leptin in 15-mo-old mice are only 20 cM apart on chromosome 13 and only exhibit their effects in middle-aged mice (15 and 18-mo-old). This suggests that the regions of chromosome 13 containing and/or flanked by these two loci may harbor genes that are capable of modulating the pace of age-related changes across multiple physiological systems. This segment of chromosome 13 bears a host of genes known to affect proliferative, regenerative, and cell death pathways. It will be of considerable interest to follow-up on these findings to identify other potential loci with significant age-related effects on multiple systems.

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DISCLOSURES

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